

PTO/PCT Rec'd 11 MAY 2001

TITLE OF THE INVENTION  
G PROTEIN-COUPLED RECEPTOR RESEMBLING THE LEUKOTRIENE B4  
RECEPTOR

5 CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

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REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

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This invention relates to a novel human DNA encoding HG07, a G protein-coupled receptor (GPCR) related to the leukotriene B4 receptor, the protein encoded by the DNA, and methods of identifying selective agonists and antagonists of the protein encoded by the DNA.

20 BACKGROUND OF THE INVENTION

G-protein coupled receptors (GPCRs) are a very large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G-proteins. Most GPCRs function by a similar mechanism. Upon the binding of  
25 agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the  $\alpha$  subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the  $\alpha$  subunit, resulting in the disassociation of the  $\alpha$  subunit from the  $\beta$  and  $\gamma$  subunits. The freed  $\alpha$  subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the  
30 agonist. Occasionally, it is the freed  $\beta$  and  $\gamma$  subunits which transduce the agonist signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, each about 20-30 amino acids long, linked by sequences of

transmembrane domains, an extracellular amino terminus, and an intracellular carboxy terminus (Strader et al., 1994, Ann. Rev. Biochem. 63:101-132; Schertler et al., 1993, Nature 362:770-772; Dohlman et al., 1991, Ann. Rev. Biochem. 60:653-688).

5 GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, *etc.* Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For  
10 example, Table 1 of Stadel et al., 1997, Trends Pharmacol. Sci. 18:430-437, lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

Leukotriene B4 (5(S),12(R)-dihydroxy-6,14-*cis*, 8,10,-*trans*-  
15 eicosatetraenoic acid) (LTB4) is involved in various immune responses, such as defenses against infection, and is involved in the process of inflammation as well. LTB4 induces chemotactic migration (Goetzl & Pickett, 1980, J. Immunol. 125:1789-1791) and chemokinetic activity (Palmer et al., 1980, Prostaglandins 20:411-418) as well as aggregation of polymorphonuclear leukocytes (Ford-Hutchinson et al., 1980,  
20 Nature 286:264-265).

High levels of LTB4 are implicated in certain disorders involving inflammation, such as psoriasis (Degiulio et al., 1993, Ann. New York Acad. Sci. 685:614-617). LTB4 has been detected in the spinal fluid of multiple sclerosis patients (Neu et al., 1992, Acta Neurol. Scand. 86:586-587) and inhibition of the  
25 interaction of LTB4 with its receptor prevents the development of paralysis and the infiltration into the spinal chord by eosinophils in experimental allergic encephalomyelitis, a murine model of multiple sclerosis (Gladue et al., 1996, J. Exp. Med. 183:1893-1898).

Yokomizo et al., 1997, Nature 387:620-624 isolated a human cDNA  
30 encoding a receptor for LTB4 from HL-60 cells that had been stimulated to differentiate by retinoic acid. Based upon its deduced amino acid sequence (GenBank accession numbers D89078 [cDNA]; D89079 [protein]), this cDNA encodes a seven-transmembrane domain G-protein coupled receptor. Transfection of this cDNA into

the cells resulted in cells that expressed this encoded receptor and not normally responsive to

specifically bound LTB4 with a  $K_d$  similar to that of differentiated HL-60 cells. Upon exposure to LTB4, the transfected cells also displayed several functional responses characteristic of LTB4-responsive cells: an increase in intracellular calcium, D-*myo*-inositol 1,4,5-triphosphate (InsP3) accumulation, inhibition of  
5 adenylyl cyclase, and chemotaxis.

#### SUMMARY OF THE INVENTION

The present invention is directed to a novel human DNA that encodes a G-protein coupled receptor, HG07. The DNA encoding HG07 is substantially free  
10 from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. Also provided is an HG07 protein encoded by the novel DNA sequence. The HG07 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:2. Methods of expressing HG07 in recombinant systems and of identifying agonists and antagonists of HG07 are provided.

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#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the complete cDNA sequence of HG07  
(SEQ.ID.NO.:1).

Figure 2 shows the complete amino acid sequence of HG07  
20 (SEQ.ID.NO.:2).

Figure 3A-B shows the location of the HG07 open reading frame. The nucleotide sequence shown is a portion of (SEQ.ID.NO.:1). The amino acid sequence shown is (SEQ.ID.NO.:2).

Figure 4 shows the results of a Northern blot of HG07 mRNA in  
25 various human tissues.

Figure 5 shows an alignment of the amino acid sequence of HG07 with the amino acid sequence of a human leukotriene B4 receptor (SEQ.ID.NO.:3; GenBank accession number D89079, see Yokomizo et al., 1997, Nature 387:620-624).

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#### DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

"Substantially free from other proteins" means at least 90%, preferably

Thus, an HG07 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG07 proteins. Whether a given HG07 protein preparation is substantially  
5 free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

“Substantially free from other nucleic acids” means at least  
10 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, an HG07 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%,  
15 of non-HG07 nucleic acids. Whether a given HG07 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

“Functional equivalent” means a receptor which does not  
20 have exactly the same amino acid sequence as naturally occurring HG07, due to alternative splicing, substitutions, deletions, mutations, or additions, but retains substantially the same biological activity as HG07. Such functional equivalents will have significant amino acid sequence  
25 identity with naturally occurring HG07. Genes and DNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring HG07. For the purposes of this invention, naturally occurring HG07 has the amino acid sequence shown as SEQ.ID.NO.:2 and is encoded by  
30 SEQ.ID.NO.:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ.ID.NO.:1.

A polypeptide has “substantially the same biological activity” as HG07 if that polypeptide has a  $K_d$  for a ligand that is no more than 5-fold greater than the

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid).

By "isolated HG07 protein" or "isolated HG07 DNA" is meant HG07 protein or DNA encoding HG07 that has been isolated from a natural source. Use of the term "isolated" indicates that HG07 protein or DNA has been removed from its normal cellular environment. Thus, an isolated HG07 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated HG07 protein is the only protein present, but instead means that an isolated HG07 protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the HG07 protein. Thus, an HG07 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated HG07 protein." Similarly, DNA encoding HG07 that is present in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) contain it through recombinant means is an "isolated DNA encoding HG07."

One aspect of this invention is an isolated DNA comprising nucleotides encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:2. This isolated DNA is substantially free from other nucleic acids and can be either single stranded or double stranded, *i.e.*, paired with its complementary sequence. Another aspect of this invention is the identification and cloning of a cDNA which encodes HG07, a G protein-coupled receptor. This cDNA is substantially free from other nucleic acids and can be either single stranded or double stranded. The present invention provides a cDNA molecule substantially free from other nucleic acids having the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1. SEQ.ID.NO.:1 contains an open reading frame (positions 158-1,324 of SEQ.ID.NO.:1) encoding a

provides a DNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 158-1,324 of SEQ.ID.NO.:1. The present invention also provides recombinant DNA molecules comprising the nucleotide sequence of positions 158-1,324 of SEQ.ID.NO.:1.

5                   Based on its predicted amino acid sequence, the HG07 protein most likely represents a novel G-protein coupled receptor (GPCR) since the HG07 protein contains many of the characteristic features of G-protein coupled receptors (GPCRs), *e.g.*,

- 10                   (a)     seven transmembrane domains;  
                  (b)     three intracellular loops;  
                  (c)     three extracellular loops; and  
                  (d)     the GPCR triplet signature sequence.

                  Northern blot analysis (Figure 4) showed that HG07 RNA is widely expressed in humans as a transcript of about 2.4 kb, especially in  
15     cells of the immune system (peripheral blood lymphocytes (PBLs) and spleen). This argues for a role for HG07 in such immune system functions as inflammation, responses to infection, chemotaxis of lymphocytes, *etc.*

                  The novel DNA sequences of the present invention encoding HG07, in whole or in part, can be linked with other DNA sequences, *i.e.*,  
20     DNA sequences to which HG07 is not naturally linked, to form "recombinant DNA molecules" containing HG07. The novel DNA sequences of the present invention can be inserted into vectors in order to direct recombinant expression of HG07. Such vectors may be comprised of DNA or RNA; for most purposes DNA vectors are preferred. Typical  
25     vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode HG07. One skilled in the art can readily determine an appropriate vector for a particular use.

                  Included in the present invention are DNA sequences that hybridize to  
30     SEQ.ID.NO.:1 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA.

                  The hybridization is carried out at 65°C in a hybridization mixture containing

Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

- Other procedures using conditions of high stringency would include
- 5 either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

- Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of
- 10 these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

- The degeneracy of the genetic code is such that, for all but two amino
- 15 acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the HG07 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:1, but still encodes the same HG07 protein as SEQ.ID.NO.:1. Such synthetic DNAs are intended to be within the scope of the present invention. If it is
- 20 desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of HG07 protein in the host.

- Another aspect of the present invention includes host cells that have
- 25 been engineered to contain and/or express DNA sequences encoding HG07. Such recombinant host cells can be cultured under suitable conditions to produce HG07. An expression vector containing DNA encoding HG07 can be used for expression of HG07 in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as
- 30 yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey, and rodent origin, and insect cells including but not limited to, *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of HG07 and which are

ATCC CCL-21 (Human Embryonic Kidney 293T) (ATCC CCL-21)

1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and  
5 MRC-5 (ATCC CCL 171).

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of the HG07 protein because these cells express a large number of G-proteins. Thus, it is likely that at least one of these G-proteins will be able to functionally couple the signal generated by interaction of  
10 HG07 and its ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, *e.g.*, cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular  $Ca^{2+}$  levels.

Other cells that are particularly suitable for expression of the HG07  
15 protein are immortalized melanophore pigment cells from *Xenopus laevis*. Such melanophore pigment cells can be used for functional assays using recombinant expression of HG07 in a manner similar to the use of such melanophore pigment cells for the functional assay of other recombinant GPCRs (Graminski et al., 1993, J. Biol. Chem. 268:5957-5964; Lerner, 1994, Trends Neurosci. 17:142-146; Potenza &  
20 Lerner, 1992, Pigment Cell Res. 5:372-378).

A variety of mammalian expression vectors can be used to express recombinant HG07 in mammalian and other cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pCR2.1 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and  
25 pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). For expression in non-mammalian cells, various suitable expression vectors are known in the art. The choice of vector will depend upon cell type used, level of  
30 expression desired, and the like. Following expression in recombinant cells, HG07 can be purified to a level that is substantially free from other proteins by conventional techniques, *e.g.*, salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography, hydrophobic



The present invention includes HG07 protein substantially free from other proteins. The amino acid sequence of the full-length HG07 protein is shown in Figure 2 as SEQ.ID.NO.:2. Thus, the present invention includes HG07 protein substantially free from other proteins having the amino acid sequence of

5 SEQ.ID.NO.:2.

As with many receptor proteins, it is possible to modify many of the amino acids of HG07, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG07 polypeptides which have

10 amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG07. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, *e.g.*, Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells,

15 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG07. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:2 wherein the polypeptides still

20 retain substantially the same biological activity as HG07. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG07.

25 When deciding which amino acid residues of HG07 may be substituted to produce polypeptides that are functional equivalents of HG07, one skilled in the art would be guided by a comparison of the amino acid sequence of HG07 with the amino acid sequences of related proteins, *e.g.*, the human leukotriene B4 receptor disclosed in Yokomizo *et al.*, 1997, Nature 387:620-624. See also Figure 5 of the

30 present application. Such a comparison would allow one skilled in the art to minimize the number of amino acid substitutions made in regions that are highly conserved between HG07 and the related protein. Accordingly, the present invention includes embodiments where the substitutions are conservative and do not occur in

One skilled in the art would also recognize that polypeptides that are functional equivalents of HG07 and have changes from the HG07 amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, *i.e.*, minimizing the differences in amino acid sequence between HG07 and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions or insertions on the biological activity of the modified HG07 polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding HG07 and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression. Assays that could be used include simple binding assays to determine if the modified HG07 polypeptide is capable of binding the same ligands, with approximately the same affinity, as the naturally occurring HG07 protein. Alternatively, one can use functional assays such as assays for increases in intracellular calcium, D-*myo*-inositol 1,4,5-triphosphate (InsP<sub>3</sub>) accumulation, inhibition of adenylyl cyclase, or chemotaxis. Such assays can be based upon similar assays for the leukotriene B<sub>4</sub> receptor disclosed in Yokomizo *et al.*, 1997, *Nature* 387:620-624.

The present invention also includes C-terminal truncated forms of HG07, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also includes chimeric HG07 proteins. Chimeric HG07 proteins consist of a contiguous polypeptide sequence of HG07 fused in frame to a polypeptide sequence of a non-HG07 protein. For example, the N-terminal domain and seven transmembrane spanning domains of HG07 fused at the C-terminus in frame to a G protein would be a chimeric HG07 protein.

The present invention also includes HG07 proteins that are in the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, *J. Biol. Chem.* 271, 16384-16392; Ng *et al.*, 1996, *Biochem. Biophys. Res. Comm.* 227, 200-204; Romano *et al.*, 1996, *J. Biol. Chem.* 271, 28612-28616).

The present invention also includes isolated forms of HG07 proteins.

The specificity of binding of compounds having affinity for HG07 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from such cells. Expression of the cloned receptor and screening for compounds that bind to HG07 or that inhibit the binding of a known  
5 ligand of HG07 to such cells, or membranes prepared from such cells, provides an effective method for the rapid selection of compounds with high affinity for HG07. Such ligands or compounds can be radiolabeled, but can also be nonisotopic compounds that can be used to displace bound radiolabeled ligands or that can be used as activators in functional assays. Compounds identified by the above method  
10 are likely to be agonists or antagonists of HG07 and may be peptides, proteins, or non-proteinaceous organic molecules.

Therefore, the present invention includes assays by which HG07 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of other receptors are well known in the art and  
15 can be adapted to identify agonists and antagonists of HG07. Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of HG07 that comprises:

- (a) transfecting cells with an expression vector encoding HG07;
- 20 (b) allowing the transfected cells to grow for a time sufficient to allow HG07 to be expressed;
- (c) exposing the cells to a labeled ligand of HG07 in the presence and in the absence of the substance;
- (d) measuring the binding of the labeled ligand to HG07;
- 25 where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG07.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of  
30 protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

The present invention also includes a method for determining

substance is a potential agonist or an antagonist of HG07, where the method comprises:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- 5 (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to HG07;
- (d) comparing the amount of binding of the substance to HG07 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG07;
- 10

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG07. Determining whether the substance is an agonist or antagonist can then be accomplished by the use of functional assays such as, *e.g.*, the assay involving the use of promiscuous G-proteins described below.

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The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

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In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK<sup>-</sup>) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

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The assays described above can be carried out with cells that have been transiently or stably transfected with HG07. Transfection is meant to include any method known in the art for introducing HG07 into the test cells. For example, transfection includes calcium phosphate or

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calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing HG07, and electroporation.

Where binding of the substance or agonist to HG07 is measured, such binding can be measured by employing a labeled  
5 substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, *e.g.*, radioactively, fluorescently, enzymatically.

In particular embodiments of the above-described methods, HG07 has an amino acid sequence of SEQ.ID.NO.:2.

10 The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, *Biochem. Biophys.*  
15 *Res. Comm.* 184:260-268.

Accordingly, the present invention provides a method for determining whether a substance is capable of binding to HG07 comprising:

- 20 (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- (b) preparing membranes containing HG07 from the test cells and exposing the membranes to a ligand of HG07 under conditions such that the ligand binds to the HG07 in the membranes;
- (c) subsequently or concurrently to step (b), exposing the  
25 membranes from the test cells to a substance;
- (d) measuring the amount of binding of the ligand to the HG07 in the membranes in the presence and the absence of the substance;
- (e) comparing the amount of binding of the ligand to HG07 in the membranes in the presence and the absence of the substance where a decrease in the  
30 amount of binding of the ligand to HG07 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG07;

where HG07 has an amino acid sequence of SEQ.ID.NO.:2.

The present invention provides a method for determining

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- (b) preparing membranes containing HG07 from the test cells and exposing the membranes from the test cells to the substance;
- 5 (c) measuring the amount of binding of the substance to the HG07 in the membranes from the test cells;
- (d) comparing the amount of binding of the substance to HG07 in the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG07;
- 10 where HG07 has an amino acid sequence of SEQ.ID.NO.:2;
- where if the amount of binding of the substance to HG07 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG07.

15 As a further modification of the above-described methods, RNA encoding HG07 can be prepared, *e.g.*, by *in vitro* transcription using a plasmid containing HG07 under the control of a bacteriophage T7 promoter, and the RNA can be microinjected into *Xenopus* oocytes in order to cause the expression of HG07 in the oocytes. Substances are then

20 tested for binding to the HG07 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which HG07 agonists and antagonists may be identified by their ability to stimulate or

25 antagonize a functional response mediated by HG07. One skilled in the art would be familiar with a variety of methods of measuring the functional responses of G-protein coupled receptors (see, *e.g.*, Lerner, 1994, Trends Neurosci. 17:142-146 [changes in pigment distribution in melanophore cells]; Yokomizo et al., 1997, Nature 387:620-624 [changes

30 in cAMP or calcium concentration; chemotaxis]; Howard et al., 1996, Science 273:974-977 [changes in membrane currents in *Xenopus* oocytes]; McKee et al., 1997, Mol. Endocrinol. 11:415-423 [changes in calcium concentration measured using the aequorin assay]; Offermanns & Simon,

1995, J. Biol. Chem. 270:15175, 15180 [changes in inositol phosphate levels]).

Accordingly, the present invention provides a method of identifying agonists and antagonists of HG07 comprising:

- 5 (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- (b) exposing the test cells to a substance that is suspected of being an agonist or an antagonist of HG07;
- (c) measuring the amount of a functional response of the  
10 test cells that have been exposed to the substance;
- (d) comparing the amount of the functional response exhibited by the test cells with the amount of the functional response exhibited by control cells;

wherein if the amount of the functional response exhibited by the test  
15 cells differs from the amount of the functional response exhibited by the control cells, the substance is an agonist or antagonist of HG07;

where the control cells are cells that have not been transfected with HG07 but have been exposed to the substance or are test cells that have not been  
exposed to the substance;

20 where HG07 has the amino acid sequence SEQ.ID.NO.:2.

In particular embodiments, the functional response is selected from the group consisting of: changes in pigment distribution in melanophore cells; changes in cAMP or calcium concentration; chemotaxis; changes in membrane currents in *Xenopus* oocytes; and changes in inositol phosphate levels.

25 HG07 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the  $G\alpha$  subunit of the G-protein to disassociate from the  $G\beta$  and  $G\gamma$  subunits. The  $G\alpha$  subunit can then go  
30 on to activate a variety of second messenger systems.

Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to ensure that the proper G-protein is present in the system

G-proteins that are "promiscuous." These promiscuous G-proteins will couple to, and thus transduce a functional signal from, virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem. 270:15175, 15180 (Offermanns). Offermanns described a system in which cells are  
5 transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins  $G\alpha 15$  or  $G\alpha 16$ . Upon the addition of an agonist of the GPCR to the transfected cells, the GPCR was activated and was able, via  $G\alpha 15$  or  $G\alpha 16$ , to activate the  $\beta$  isoform of phospholipase C, leading to  
10 an increase in inositol phosphate levels in the cells.

Therefore, by making use of these promiscuous G-proteins as in Offermanns, it is possible to set up functional assays for HG07, even in the absence of knowledge of the G-protein with which HG07 is coupled *in vivo*. One possibility is to create a fusion or chimeric protein composed of  
15 the extracellular and membrane spanning portion of HG07 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the HG07 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of HG07 comprising:

- 20 (a) providing cells that expresses a chimeric HG07 protein fused at its C-terminus to a promiscuous G-protein;
- (b) exposing the cells to an agonist of HG07;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG07;
- 25 (d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG07.

Another possibility for utilizing promiscuous G-proteins in  
30 connection with HG07 includes a method of identifying agonists of HG07 comprising:

- (a) providing cells that expresses both HG07 and a promiscuous G-protein:



(b) exposing the cells to a substance that is a suspected agonist of HG07;

(c) measuring the level of inositol phosphates in the cells; where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of HG07.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK<sup>-</sup>) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG07 and the promiscuous G-protein in the cells.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of G $\alpha$ 15 or G $\alpha$ 16. Expression vectors containing G $\alpha$ 15 or G $\alpha$ 16 are known in the art. See, *e.g.*, Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J. Biol. Chem. 268:10139-10144.

The above-described assay can be easily modified to form a method to identify antagonists of HG07. Such a method is also part of the present invention and comprises:

- (a) providing cells that expresses both HG07 and a promiscuous G-protein;
- (b) exposing the cells to a substance that is an agonist of HG07;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG07;
- (d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG07.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK<sup>-</sup>) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG07 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of G $\alpha$ 15 or G $\alpha$ 16.

In particular embodiments of the above-described methods, HG07 has an amino acid sequence of SEQ.ID.NO.:2.

Another functional assay that can be used to identify agonists and antagonists of HG07 relies on the ability of leukotriene B4 to induce the chemotaxis or chemokinesis of certain cell types through action at the leukotriene receptor (see, 5 *e.g.*, Yokomizo et al., 1997, *Nature* 387:620-624; Ng et al., 1991, *J. Immunol.* 147:3096-3103). Given the high degree of similarity in amino acid sequence between HG07 and the leukotriene B4 receptor (about 46%, see Figure 5), it is expected that agonists of HG07 will be able to induce the chemotaxis or chemokinesis 10 of cells.

Accordingly, the present invention provides a method of identifying agonists of HG07 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG07 in the cells;
- 15 (b) exposing the test cells to a substance;
- (c) measuring the amount of chemotaxis or chemokinesis exhibited by the test cells that have been exposed to the substance;
- (d) comparing the amount of chemotaxis or chemokinesis exhibited by the test cells with the amount of chemotaxis or chemokinesis 20 exhibited by control cells;

wherein if the amount of chemotaxis or chemokinesis exhibited by the test cells is greater than the amount of chemotaxis or chemokinesis exhibited by control cells, the substance is an agonist of binding to HG07;

where the control cells are cells that have not been transfected with 25 HG07 but have been exposed to the substance or are test cells that have not been exposed to the substance;

where HG07 has the amino acid sequence SEQ.ID.NO.:2.

In particular embodiments the cells are CHO cells.

While the above-described methods are explicitly directed to testing 30 whether "a" substance is an agonist or antagonist of HG07, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of HG07. Accordingly, the use of collections of

Agonists and antagonists of HG07 that are identified by the above-described methods are expected to have utility in the treatment of diseases that involve the inappropriate expression of HG07. In particular, given the expression pattern of HG07 (see Figure 4), such agonists and antagonists are expected to have utility in the treatment of various immune system disorders or disorders involving inflammation, *e.g.*, multiple sclerosis, arthritis, asthma, lupus. Such agonists and antagonists are also expected to be useful in treating various infectious diseases.

Given the resemblance between HG07 and the leukotriene B<sub>4</sub> (LTB<sub>4</sub>) receptor, it is expected that agonists and antagonists of HG07 will have pharmacological activity and be useful in a manner similar to that in which agonists and antagonists of the LTB<sub>4</sub> receptor are useful. Agonists and antagonists of the LTB<sub>4</sub> receptor are useful in the treatment of rheumatoid arthritis, gout, psoriasis, inflammatory bowel disease (U.S. Patent No. 5,684,162), chronic lung diseases, endotoxic shock, septic shock, and adult respiratory distress syndrome (U.S. Patent No. 5,552,441).

While HG07 appears to be most similar to the LTB<sub>4</sub> receptor, and thus is expected to be pharmacologically useful in a manner similar to that of the LTB<sub>4</sub> receptor, it is also expected that HG07 may have affinity for other related leukotrienes, *e.g.*, leukotrienes A<sub>4</sub>, C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub>. Antagonists of the leukotriene D<sub>4</sub> receptor, such as zafirlukast, montelukast, and pranlukast, have been shown to have utility in the treatment of asthma (Tan & Spector, July/August 1997, Science and Medicine 26-33). Thus, it is expected that antagonists of HG07 may also have utility in the treatment of asthma.

The present invention includes pharmaceutical compositions comprising agonists and antagonists of HG07. The agonists and antagonists are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing agonists and antagonists and carriers can be found in Remington's

composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the agonists and antagonists.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where  
5 HG07 activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, gender, and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages.  
10 Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms  
15 as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or  
20 intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be  
25 administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent  
30 throughout the dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be

function of the patient; and the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving

5 concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

The present invention also includes methods of expressing

10 HG07 in recombinant systems and then utilizing the recombinantly expressed HG07 for counter-screening. When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is

15 necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find compounds that are potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological

20 effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, *etc.* (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). Therefore, HG07 proteins and DNA

25 encoding HG07 proteins have utility in counter-screens. That is, they can be used as "minus targets" in counter-screens in connection with screening projects designed to identify compounds that specifically interact with other G-protein coupled receptors.

The DNA of the present invention, or hybridization probes

30 based upon the DNA, can be used in chromosomal mapping studies in order to identify the chromosomal location of the HG07 gene or of genes encoding proteins related to HG07. Such mapping studies can be carried out using well-known genetic and/or chromosomal mapping techniques

markers or *in situ* hybridization. See, e.g., Verma et al., 1988, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY. After identifying the chromosomal location of the HG07 gene or genes encoding proteins related to HG07, this information can be

5 compared with the locations of known disease-causing genes contained in genetic map data (such as the data found in the genome issue of Science (1994, 265:1981-2144). In this way, one can correlate the chromosomal location of the HG07 gene or of genes encoding proteins related to HG07 with the locations of known disease-causing genes and thus help to limit

10 the region of DNA containing such disease-causing genes. This will simplify the process of cloning such disease-causing genes. Also, once linkage between the chromosomal location of the HG07 gene or of genes encoding proteins related to HG07 and the locations of a known disease-causing gene is established, that linkage can be used diagnostically to

15 identify restriction fragment length polymorphisms (RFLPs) in the vicinity of the HG07 gene or of genes encoding proteins related to HG07. Such RFLPs will be associated with the disease-causing gene and thus can be used to identify individuals carrying the disease-causing gene.

For such chromosomal mapping studies as described herein,

20 it may be advantageous to use, in addition to the DNA of the present invention, the reverse complement of the DNA of the present invention or RNA corresponding to the DNA of the present invention.

Nucleotide sequences that are complementary to the HG07 sequences disclosed herein can be synthesized for use in antisense

25 therapy. Such antisense molecules can be DNA, stable derivatives of DNA such as phosphorothioates or methyl phosphonates, RNA, stable derivatives of RNA such as 2'-O-alkyl RNA, or other forms of HG07 antisense molecules. HG07 antisense molecules can be introduced into cells by a variety of methods, e.g., microinjection, liposome encapsulation,

30 or by expression from vectors harboring the antisense sequence. HG07 antisense therapy is expected to be particularly useful in the treatment of conditions where it is beneficial to reduce HG07 activity.

The present invention also includes antibodies to the HG07

antibodies and are useful in treating disorders of the immune system that involve the inappropriate expression or activity of the HG07 protein. The antibodies of the present invention are raised against the entire HG07 protein or against suitable antigenic fragments of the protein that are

5 coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the

10 Biosciences) 4:181-186.

For the production of polyclonal antibodies, HG07 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of

15 antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, HG07 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case

20 of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce HG07 polypeptides into the cells of target organs. Nucleotides encoding HG07 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes

25 virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding HG07 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures

30 and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene



therapy with HG07 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate HG07 activity.

A cDNA fragment encoding full-length HG20 can be isolated from an appropriate human cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for HG20 shown in Figure 1 as SEQ.ID.NO.:1. Suitable primer pairs would be, *e.g.*:

CGCGGATCCGCCATCATGGCACCTTCTCATCGG  
(SEQ.ID.NO.:4 and  
GCGGGATCCTCAAAGGTCCCATTCGG (SEQ.ID.NO.:5).

The above primers contain BamHI sites in their 5' ends to facilitate cloning of the amplified cDNA into suitable vectors, *e.g.*, pcDNA3.1. The above primers are meant to be illustrative. One skilled in the art would recognize that a variety of other suitable primers can be designed.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl<sub>2</sub>, 200  $\mu$ M for each dNTP, 50 mM KCl, 0.2  $\mu$ M for each primer, 10 ng of DNA template, 0.05 units/ $\mu$ l of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding HG07 can be isolated would be a human cDNA library made from RNA from prostate, ovary, pancreas, peripheral blood lymphocytes, or spleen. Such libraries can be prepared by methods well-known in the art. Alternatively, several commercially available libraries would be suitable, *e.g.*, cDNA libraries from Stratagene, Inc., La Jolla, CA, USA, such as human ovary, catalog #937217, human pancreas, catalog #937208, or human fetal spleen, catalog #937205). The primary clones of such

By this method, a cDNA fragment encoding an open reading frame of 389 amino acids (SEQ.ID.NO.:2) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, Ca). HG07 protein can then be produced by transferring an expression vector encoding HG07 into a suitable host cell and growing the host cell under appropriate conditions. HG07 protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding HG07 can be isolated from a cDNA library using as a probe oligonucleotides specific for HG07 and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, *e.g.*, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for HG07 and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of HG07 shown in Figure 1 as SEQ.ID.NO.:1 and can be synthesized by methods well-known in the art.

Genomic clones containing the HG07 gene can be obtained from commercially available human PAC or BAC libraries, *e.g.*, from Research Genetics, Huntsville, AL. Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the HG07 can be isolated, using probes based upon the HG07 nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).

The following non-limiting examples are presented to better illustrate the invention.

30

## EXAMPLE 1

### Tissue distribution of HG07 RNA transcripts

hybridized with  $^{32}\text{P}$ -labelled probes prepared from a 805 bp BamHI-ApaI fragment of HG07. Hybridizations were carried out using the ExpressHyb buffer supplied by Clontech under conditions suggested by Clontech. The blots were then washed in 0.1X SSC/0.1% SDS at 60°C and exposed by autoradiography.

5

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

10

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.